THE CHLOROPHYLL CONTENT OF LEAVES OF THE COMMON BEAN

Phaseolus vulgaris, L

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Warren Albert Ruth

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BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy

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I Introduction

From the standpoint of plant and animal nutrition chlorophyll is of the utmost importance. To its quantitative relations and specific function in plants is to be attributed the faculty of the plant to produce much or little organic material under the prevailing conditions. Our knowledge does not enable us to make a definite statement concerning the difference in chlorophyll in different individuals or species. The results of recent and earlier investigations would lead us to the belief that the difference in performance, that is, in the ability to produce organic matter, rests largely, if not entirely, with the protoplast and the quantity, rather than the quality of chlorophyll.

Rose (1913, p. 29) compared leaf extracts with a standard extract of unknown value. "La chlorophylle n'ayant pas été isolée, on ne peut, comme le fait observer Lubimenko, procéder à des déterminations de quantités absolues. Il faut se contenter de la détermination de quantités relatives à un étalon". Not until Willstätter and Stoll (1913) published a method of analysis could the absolute quantity of chlorophyll per unit area of leaf tissue be determined.

The present study was undertaken to determine the quantity of chlorophyll per unit area of leaf surface in plants growing under different conditions. The plans for a systematic quantitative study of the chlorophyll content of foliage leaves, as here outlined, is part of a general project of the laboratory of plant physiology

I Introduction

of the University of Illinois. The studies are to be prosecuted along two general lines, - First, a study of the quantitative relation of the chlorophyll content of plants, treated and untreated, growing under conditions such as practical greenhouse men now advise for vegetable growing. The conditions for successful greenhouse culture, in the main, approach very closely the ecological optimum (Schimper, 1903,p.160). Second, a study of the chlorophyll content of leaves as affected by the shifting of one or more of the ecological factors, as light, temperature, or moisture, independently or together toward plus or minus, etc. The second plan involves long and difficult experimentation if the results are to be of permanent value. It becomes necessary to provide for the strict regulation of each environmental factor or factors working together or in opposition . The variation in effect of a single factor under widely varied conditions has been clearly brought out by Livingston (1917,[1] p. 6). "Less than twenty-five years ago it was still taught in physiological laboratories that the relation of a given process (such as respiration, absorption, and so forth) to the intensity of any evironmental or internal condition (such as temperature, concentration of solutions, and so forth) might be established by maintaining all the other conditions constant and causing the intensity of the condition under investigation to vary from experiment to experiment. No attention was apparently to be given to the intensities of the other conditions; they were merely to be constant, or were to vary in the same manner for all comparable experiments. With more knowledge and with deeper thinking we have become aware that the constellation or complex of other conditions frequently

exerts a pronounced influence upon the effect produced in the organism by a given difference in the intensity of the one condition studied.----------Failure to realize this, failure to measure and describe all the other effective conditions - which were not thought of as being elements of the problem in hand - has frequently given rise to more or less serious polemics between different experimenters, and so to waste of time, energy, and accumulated wealth." The work reported in this paper is based on the first method as described above.

I wish to thank Professor Charles F. Hottes, of the department of botany of the University of Illinois, for his advice and help given me at every stage of my work.

II Materials and Methods

Plants of Phaseolus vulgaris (Var. Dreer's Extra Early Refugee) were used in all experiments. The seed was obtained in one lot from Vaughan's Seed Store, Chicago, Illinois. The plants were grown in the greenhouse, in a uniformly mixed and sifted Urbana brown silt-loam, in flats three inches deep, ten and one-half inches wide, and twenty-two and one-half inches long, internal measurements.

In planting, the slightly moist soil was sifted into the flats uniformly to within one inch of the top. The beans were laid on this soil two inches apart in the row and with four inches between the rows. One inch of soil was then added, smoothed off, and evenly compacted. The flats were then watered and covered un-

til the seedlings appeared above the soil. The flats were then transferred to rotating tables as described by Livingston (1917[2], p. 149).

As soon as the primary leaves had unfolded, from twelve to twenty-four plants of uniform appearance and growth were selected, attention at the same time being paid to spacing, an equal number of plants was left in each half of the flat. One-half of the plants of each flat were treated, the other half serving as a check for comparison. In the experiments on the effect of spraying with Bordeaux mixture the plants in one-half of a flat were sprayed; in the experiments on the effect of the removal of the growing bud, alternate plants. In the experiments on the effect of age, care was taken to maintain an equal spacing as plants were removed during the progress of the experiment.

The rotating tables (Livingston,1917[2]) were located in the center of a south room in the greenhouse. The tripods were set on widely spaced two inch boards, which were supported on a solid frame made of iron pipes. The nature of the foundation and the height of the platforms above the ground (four feet) secured a free circulation of air between the flats, and together with the distance from the heating pipes (six feet) guarded against uneven heating and uneven relative humidity. Power was transmitted from the motor, which was supported on a separate stand, to the tables by a one-quarter inch leather belt. Locating the tables and motor on separate stands reduced vibration. The peroid of rotation of the tables was once in six and one-half minutes.

The steam heat of the greenhouse was under thermostatic

control, maintained at twenty degrees C. The humidity of the air was controlled by flooding the broad trenches under the slatted walks and benches each morning. At this time the flats, also, were watered. On cloudy days the tables were artificially illuminated by stage flood-lights, each provided with a 1000 watt nitrogen-filled tungsten lamp. The distance from the lamps to the tables was five feet. The effectiveness of this additional illumination as an aid to the normal growth of the plants (Lubimenko, 1905) is indicated by the prevention of unusual elongation of the hypocotyl on such days. The satisfactory results obtained by this artificial lighting may be illustrated by the following: - December 13, 14, and 15, were very cloudy. On December 13, the cotyledons of the young plants were just appearing above the soil of two flats. One of these was artificially illuminated by means of a flood-light; the other, similarly located, received daylight only. The length of the hypocotyl of the leaves of the beans in the former on December 16, averaged 9 cm. (individual measurements, 10, 9, 13, 10, 9, 9, 6, 6, 6, 12, 9, 6, 11, 9, and 9 cm.), while those of the latter averaged 15 cm. (individual measurements, 16, 16, 13, 16, 15, 14, and 16). (Johnston, 1917). These plants were not used.

The results herein recorded are based upon data secured through the use of primary leaves of the bean plant. These were measured, weighed, and immediately dried. No selection of plants or leaves was made at this time. The area of the leaves was determined by drawing the outline on letter-size Hammermill bond, and weighing. The paper was found remarkably uniform in weight; nevertheless, each sheet was separately weighed. The leaves were

rapidly dried in circulating air at a temperature of forty-five degrees C. to fifty degrees C. About an hour was required for average-sized leaves. During drying, the leaves were protected from sunlight. As soon as the leaves were crisp they were stored in small tin boxes until their chlorophyll content was determined.

In determining the chlorophyll content of the dried leaves, Willstätter's methods were used, with very slight modifications. The chlorophyll in all cases was extracted with eighty-five percent acetone. The powder of treated leaves as well as those of the checks grown at the same time and under identical conditions was extracted and compared for its chlorophyll content with an extract similarly prepared, but from a leaf powder standardized for its chlorophyll content in a manner to be described below.

The standardized solutions were prepared as follows: - The primary leaves of a large number of young bean plants, grown as already described and normal in every respect, were dried and ground to pass a 100-mesh sieve. Preliminary experiments showed that this powder gave, on extraction with 50 cc. of eighty-five percent acetone, a solution suitable for use in the Duboscq colorimeter. The preparation of the standard chlorophyll extract for the colorimeter and for the determination of the absolute chlorophyll content of the leaves was done with extreme care. The entire amount of leaf powder was first thoroughly mixed, then quartered, and from these portions small composite samples were taken and weighed. The quantity of the powder used for the color standard was .25 gram; for the absolute chlorophyll determination, 2.5 grams. The extraction of each .25 gram of powder was accomplished by allowing it to stand for two hours

in contact with 20 cc. of eighty-five percent acetone. The extract was filtered through a 7 cm. Buchner funnel. After disconnecting the flask from the suction pump, the beaker and residue were rinsed with a few centimeters of the eighty-five percent acetone, until the filtrate came through colorless. The filtrate, after it had been transferred to a 50 cc. volumetric flask and made up to the mark with eighty-five percent acetone, served as the standard solution. The flask was kept tightly stoppered and in the dark except when a part of the solution was removed for use in the colorimeter.

Each lot of air-dried leaves from treated plants and their corresponding checks was weighed and ground, and two .25 gram samples were immediately taken from each lot and extracted in 50 cc. of eighty-five percent acetone in the manner described. The chlorophyll content of the respective extracts was determined by comparison with the standard solution using a Duboscq colorimeter with the standard set at 20 mm.

Standardization of the leaf powder was accomplished by using Willstätter's method for absolute chlorophyll determination. The essential steps in the method are (1) transferring the pigments in the raw extract to ether; (2) saponification of the two chlorophylls with methyl alcoholic potash; (3) the separation of these products from carotin and xanthophyll by extracting them with water; (4) their colorimetric comparison with a solution containing a weighed amount of pure chlorophyll.

The pure chlorophyll was isolated from the same leaf-powder to insure the proper proportion of chlorophylls a and b. Ten-gram samples were used for this purpose. The principal steps are:-

(1) complete extraction of the leaf-powder with eighty-five percent acetone; (2) transference of the pigments to petrol-ether; (3) removal of the greater part of the acetone by washing with water; (4) separation of xanthophyll by washing with eighty percent methyl alcohol; (5) removal of acetone and methyl alcohol by washing with water, - which results in the precipitation of the chlorophyll; (6) removal of the water by means of anhydrous sodium sulphate; (7) removal of the fine precipitate of chlorophyll and its separation from carotin by filtering through talc; (8) solution of the precipitated chlorophyll in ether and reprecipitation by adding petrol ether; (9) filtration through talc; (10) solution of the precipitate in ether; (11) evaporation to dryness. A lengthy description of this method is found in Willstätter and Stoll, 1913, (pp. 80-82 and 132-135).

The only modification of Willstätter's methods as described in the reference above was the use of comparatively larger quantities of solvent. This was because comparatively small amounts of leaf-powder were extracted. On this account, Willstätter's first dilution of the eighty-five percent acetone extract was omitted in the process of standardization of the leaf-powder, and in the extraction and separation of pure chlorophyll, larger comparative volumes of all the solvents were used than Willstätter recommends.

Before the spraying experiments were started enough Bordeaux mixture (at the usual rate of four pounds of lime and four pounds of copper sulphate in fifty gallons) was made up to last until the experiments were discontinued. The lime, made from a high grade marble, was thoroughly slaked by adding boiling distilled water and boiling until a smooth cream was formed; it was then diluted to one-

half the final volume of the spray. The copper sulphate (Bakers, C. P.) was dissolved in hot distilled water and made up to an equal volume. After cooling, the ingredients were poured at an equal rate into another container. The mixture was shaken for seventy-two hours at the temperature of the laboratory before any was used. (Bell and Taber, 1907). An atomizer was used in spraying the plants. The spray was applied only to the upper surface. Whenever Bordeaux mixture was applied, distilled water was applied to the corresponding checks.

III The Effect of Bordeaux Mixture

A considerable part of the time of experimentation was devoted to a study of the effect of spraying the primary leaves of the beans with Bordeaux mixture. It is a well established fact that a large number of plants sprayed with this fungicide show a distinctly greener color. This would lead one to expect an increase in the chlorophyll content of sprayed leaves. The deeper green of sprayed as contrasted with unsprayed leaves may be due to an increase in quantity of chlorophyll in the plastids, or to an increase in plastids, or both, or to the lack of growth of the leaf and cells and consequently to the relative crowding of the normal number of plastids in a smaller area. To my knowledge no quantitative experiments have been made to measure the increase in chlorophyll content of sprayed leaves should such be the case.

The physiological effect of Bordeaux mixture on most plants is undoubted, and has been observed in one or another of its

manners of manifestation since the discovery of this fungicide. (Millardet and Gayon, 1885[1]). That the effects of Bordeaux mixture are due to the copper ion has been the conclusion of most, but not all, investigators. Nägeli (1893) found that water in a glass vessel in which copper coins had been placed had a toxic effect upon Spirogyra. He attributed this to an action without contact, which he called "oligodynamic". Frank (1888) found that the water from a copper still had a toxic effect upon the roots of lupines. Loew (1893) attributed the facts observed by Nägeli and Frank to traces of copper in solution. Millardet and Gayon (1885[2]) found that the normal germination of spores of Peronospora viticola would not take place at a higher concentration of a copper salt than three parts in ten million. They found later (Millardet and Gayon 1887) that the cuticle of leaves had the power of removing copper from a solution of copper sulphate. They believed that the copper absorbed in this way actually penetrated the cells of leaves sprayed with Bordeaux. Bain's very careful investigations (1902) have sustained this belief. Bain (1902, p. 88) states his conclusions as follows: - "From all the evidence presented on the preceeding pages, there can remain little doubt that copper is absorbed by the leaves of plants sprayed with Bordeaux mixture.----------Of course, the most conclusive and only final evidence of the entrance of the copper into the tissues of the leaf is to demonstrate its presence there by an appropriate test. The writer has made no experiments in this direction." The belief of Millardet and Gayon in actual penetration of copper (1887) receives further support from the work of Schander (1904) who believed, however, that the action of rain and dew was seldom of direct impor-

tance in dissolving copper salts from the precipitated Bordeaux mixture because of its insolubility, and that in many plants, the entrance of copper occurred through the hairs, e.g., the bean and apple, as the result of their excretion of alkaline substance, or, in the case of other plants like Fuchsia and Oenothera, through glands, following the excretion of acidic substances. Crandall (1902, p. 230) objects to the conclusion of Millardet and Gayon in regard to penetration, because of the large amounts of soluble copper they used in their experiments, and the insolubility of the Bordeaux precipitate. Rumm (1893) because he was unable to find traces of copper in leaves affected physiologically by spraying with Bordeaux mixture, and Frank and Krüger (1894) because they thought too little copper was dissolved in the filtrate from Bordeaux to act on fungous spores, applied Nägeli's hypothesis of oligodynamic action to this case. Duggar and Bonns (1918), in a study of the effects of spraying with Bordeaux mixture on transpiration, offer as an explanation of its effect on this particular process the following: - The Bordeaux mixture, which Duggar and Cooley (1914) regard as a film, acts as a bibulous material, taking water directly from the interior of the plant. This explanation is based on the facts, as observed by them, that xerophytic plants show no increased transpiration when sprayed, and that the increase occurs, in the case of mesophytes, at night only, at which time the stomata are probably suffused with water. If this explanation of Duggar and Bonns should be proven correct, then the color change of sprayed leaves must be dependent upon a change in the rate of transpiration. The manner of action of Bordeaux mixture has remained unsolved. The failure to realize the

fundamental fact that while the concentration of copper in solution in Bordeaux mixture is small, the abundant precipitate assures a constant concentration of copper in solution, as long as this precipitate remains unchanged in character, has possibly delayed the solution of the problem. As a part of the copper is removed, by penetration or otherwise, an equal quantity of copper passes into solution. Concentration does not play the part in toxicity or other physiological effect of Bordeaux mixture that it plays in the case of a solution not in contact with the solid phase of the salt in solution, where concentration, as Clark (1902) has shown, is among the important conditions. That the physiological effects of Bordeaux mixture are due to the penetration of copper in solution seems still more probable if we consider the results of Kahlenberg and True (1896) and Heald (1896) in connection with certain of those of Bain (1902). The former authors, working with seedlings in very dilute solutions of copper salts, proved that the toxicity of the dissolved copper salt was directly proportional to the concentration of copper ions in the solution. Bain (1902, pp. 44-52) showed that sprayed foliage is not injured unless liquid water is present on the leaf. He (1902, pp. 36-44) showed also that the salts which one would expect to increase the concentration of ions in Bordeaux solutions, for example, calcium chloride, calcium sulphate, and calcium nitrate, produce an increased Bordeaux injury, while lime and various carbonates, which could be expected to reduce the concentration of copper ions, prevent Bordeaux injury. Besides the change in the concentration of copper ions the antagonism of copper and calcium, which has been proven by True and Gies (1903) and Hawkins (1913), may have

been involved in Bain's experiments. As a result of the work of Boussingault (1878) and Bain (1902) there can be little doubt that relatively insoluble calcium salts, such as those found in Bordeaux mixture, can penetrate the leaf.

It is desirable, in a paper dealing with the physiological effects of Bordeaux mixture on chlorophyll formation, to review briefly the known physiological effects of copper. Many instances have been found of the accelerating affect of dilute solutions of toxic agents upon growth. Coupin's results (1898) proved that comparatively dilute sea-water and sodium chloride accelerated the growth rate of certain plants. True and Gies (1903) obtained a similar effect upon the growth of the roots of Lupinus albus, brought about by dilute solutions of copper salts. When sufficiently dilute, a more or less clearly marked stimulating effect was observed upon the growth of the primary radicles. When at a greater concentration, perhaps double that causing stimulation, a retarding influence was usually seen, and in a concentration approximately double this, growth was much interfered with. On again doubling the concentration, little or no growth took place. These results, in the very dilute solutions employed, are clearly due to the copper ion, and may be compared with those of Kahlenberg and True (1896) and Heald (1896). Jensen (1907) has shown that wheat seedlings, growing in quartz sand to which had been added small amounts of solutions of copper sulphate, manifested in sands containing a certain very small amount, an acceleration in growth and transpiration. The acceleration in growth was indicated by an increased length, total fresh weight, and total dry weight. In sands containing copper sulphate

in greater or smaller amounts, there was a concurrent decrease in transpiration and in the growth rate, as indicated by the three indices mentioned.

The great stimulus to the study of the physiological effects of copper has been, of course, the use of Bordeaux mixture as a fungicide. Galloway (1890) reported an increase in the sugar content of grapes, and an earlier ripening due to its use. Rumm (1893) reports the same effect, and a deeper color. Frank and Krüger (1894) found that spraying potato plants with Bordeaux mixture seemingly increased the chlorophyll content slightly and the assimilatory activity markedly, as shown by a comparison of the starch content of sprayed and unsprayed leaves. They found an increased yield of tubers, in one variety in the proportion of nineteen to seventeen, and in the other variety under experiment in the proportion of seventeen to sixteen. The leaf seemed to be slightly thicker and stronger, although the ground structure of the leaves was not altered. They also found that sprayed plants lived longer, and that transpiration was increased. Since that time Frank and Krüger's conclusions have been substantiated by the work of other investigators with other plants. The effect of copper upon the photosynthetic process has been studied directly by Treboux (1903) and he arrives at a conclusion that seemingly is directly opposed to the idea of an increase in the photosynthetic products - starch and sugar - expressed above. He concludes: - "Es sei hier gleich bemerkt, dass es mir trotz zahlreicher Versuche nie gelungen ist, eine andere als herabsetzende Wirkung verschiedener Gifte auf die Assimilation zu constatieren; auch in der Litteratur liegt meines Wissens

eine Angabe über eine beschleunigende Wirkung nicht vor.

"Geprüft wurden hauptsächlich folgende Stoffe: Metallgifte ($ZnSO_4$, $CuSO_4$, $CoSO_4$, $FeSO_4$, $HgCl_2$), Alkaloide (Chinin, Morphium, Cocain), Anaesthetica (Aether, Chloroform, Alkohole) und Methylenblau (s. Vers. VII-XVII).

"Stärkere Concentrationen hoben die Assimilation sofort auf; beim Herabgehen auf schwächere nahm dann die schädigende Wirkung allmählich ab und unterblieb schliesslich ganz, wenn die Concentration den Grenzwerth der Giftigkeit erreichte, ging aber nie in eine beschleunigende über. Die Stärke der Wirkung entsprach bei vergleichbaren Stoffen im Allgemeinen der Schädlichkeit für die Pflanzenzelle; so setzten von den Metallgiften $HgCl_2$ und $CuSO_4$ die Assimilation am starksten herab."

For further literature the reader is referred to Lutman (1916).

The results obtained by spraying with Bordeaux mixture may be indicated by reporting the following experiment: - The primary leaves of one-half of the plants in three flats were sprayed as soon as the leaves had fully unfolded; these leaves represented the stage of development indicated below as "Age A". At that time the average length of the leaves to be sprayed was 4.6 cm.; the leaves to be left unsprayed also averaged 4.6 cm. The measurements are given in detail in Table Ia. The flats are numbered 1, 2, and 3.

Table Ia

Length (in Centimeters) of Primary Leaves of Plants Just Before Spraying; Flats 1, 2, and 3.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
1	1	4.2	4.5	4.4	1	5.1	5.2	5.2
	2	4.5	3.2	3.9	2	5.3	5.3	5.3
	3	5.3	5.5	5.4	3	5.6	5.7	5.7
	4	5.2	5.5	5.3	4	4.6	4.5	4.5
	5	4.1	4.2	4.2	5	4.0	4.3	4.2
	6	5.2	5.1	5.1	6	2.8	2.7	2.8
	7	abnormal			7	3.5	3.0	3.2
	Average.....4.7				Average.....4.4			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
2	1	5.2	5.3	5.2	1	3.6	3.8	3.7
	2	4.3	4.6	4.5	2	5.3	5.2	5.2
	3	4.8	4.6	4.7	3	4.8	4.5	4.6
	4	3.5	3.7	3.6	4	3.5	3.3	3.4
	5	3.8	3.5	3.7	5	5.3	5.6	5.4
	6	5.5	5.4	5.4	6	5.5	5.7	5.6
	7	3.8	4.0	3.9	7	4.8	4.7	4.8
	8	5.7	5.6	5.6	8	4.9	5.0	4.9
	Average.....4.8				Average.....4.7			
3	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
	1	4.3	4.5	4.4	1	4.6	4.5	4.6
	2	4.9	5.0	4.9	2	5.1	5.0	5.1
	3	5.0	5.1	5.1	3	4.7	4.8	4.7
	4	2.8	3.3	3.1	4	4.4	4.5	4.5
	5	4.4	4.4	4.4	5	4.7	5.0	4.8
	6	5.5	5.0	5.2	6	4.6	4.7	4.6
	Average.....4.5				Average.....4.7			
Average; Flats 1 - 3 .....4.6					Average.....4.6			

Four days later the primary leaves of three other flats were sprayed. These flats had been planted when flats 1, 2, and 3, were planted, and received exactly the same treatment except that spraying was deferred four days. The average size of the leaves to be sprayed was, for the three flats, 6.86 cm.; the average size of the leaves not to be sprayed was 7.19 cm. This stage of development is designated below as "Age B". The flats are numbered 4, 5, and 6. Individual measurements are given in Table Ib.

Table Ib

Length (in Centimeters) of Primary Leaves of Plants Just Before Spraying; Flats 4, 5, and 6.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
4	1	5.7	4.3	5.0	1	7.3	7.3	7.3
	2	5.6	5.8	5.7	2	7.9	7.2	7.5
	3	5.7	6.3	6.0	3	6.2	5.3	5.8
	4	4.3	4.2	4.3	4	6.3	6.3	6.3
	5	8.4	9.0	8.7	5	7.8	7.7	7.7
	6	6.4	6.4	6.4	6	4.2	3.8	4.0
	7	7.6	7.7	7.6	7	6.6	6.8	6.7
	Average.....6.2				Average.....6.5			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
5	1	6.2	7.3	6.8	1	8.9	8.3	8.6
	2	8.0	8.5	8.2	2	7.0	7.9	7.4
	3	7.3	6.9	7.1	3	8.5	8.8	8.6
	4	6.8	6.2	6.6	4	6.8	6.7	6.8
	5	7.5	7.3	7.4	5	8.4	8.6	8.5
	6	9.4	9.5	9.4	6	7.8	7.1	7.5
	7	7.3	8.0	7.7	7	7.2	7.5	7.4
	Average.....7.6				Average.....7.8			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
6	1	7.0	7.0	7.0	1	--	--	--
	2	6.8	7.5	7.1	2	5.8	6.0	5.9
	3	6.6	6.4	6.5	3	8.3	7.6	7.9
	4	6.3	6.2	6.3	4	7.8	6.7	7.3
	5	7.2	7.7	7.4	5	8.2	8.7	8.4
	6	6.3	5.9	6.1	6	7.1	6.7	6.9
	Average.....6.7				Average.....7.3			
Average; Flats 4 - 6 .....6.9					Average.....7.2			

The average length of the primary leaves to be sprayed was, for the six flats, 5.78 cm.; that of the primary leaves to be left as checks was 5.83 cm.

The plants were harvested and records were made of the area and weight of the primary leaves of the plants of all six flats on the same day. This was three days after spraying flats 4, 5, and 6, and one week after spraying flats 1, 2, and 3. At this time the secondary leaves averaged about 5 cm. in length, and did not shade, to any extent, the primary leaves. This stage of development is represented below as "Age C."

The figures presented in the text to follow are averages of measurements made on individual plants, with the exception of the chlorophyll content per plant. The primary leaves of plants in halves of flats were analyzed together, and the chlorophyll per plant was obtained by dividing the result by the number of plants in each half-flat.

Effect of Bordeaux mixture upon area. - At harvesting, the primary leaves of sprayed plants were smaller than the primary leaves of the unsprayed plants of the same flat, with the exception of those of the sprayed and unsprayed plants grown in Flat 3. The results are shown in Tables IIa and IIb.

Table IIa

Area (in Square Centimeters) of Sprayed and Unsprayed Primary Leaves of Flats 1, 2, and 3, Seven Days After Spraying.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
1	1	40.0	58.2	49.1	1	58.0	56.7	57.4
	2	40.0	56.8	48.4	2	80.2	84.3	82.3
	3	51.7	82.5	67.1	3	55.4	62.3	58.9
	4	51.1	62.2	56.6	4	63.7	62.3	63.0
	5	59.5	59.5	59.5	5	58.0	60.8	59.4
	6	52.5	60.7	56.6	6	51.1	51.1	51.1
	7	20.8	33.2	27.0	7	69.2	83.0	76.1
	Average.....52.0				Average.....64.0			
	Difference (%)..... -18.8							
2	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
	1	62.2	66.4	64.3	1	38.7	37.3	38.0
	2	69.1	59.5	64.3	2	69.4	69.4	69.4
	3	70.8	75.0	72.9	3	71.8	71.8	71.8
	4	24.4	23.0	23.7	4	34.4	37.3	35.8
	5	34.4	38.7	36.5	5	78.6	82.8	80.7
	6	60.0	57.2	58.6	6	82.8	81.5	82.1
	7	37.2	37.2	37.2	7	70.0	57.2	63.6
	8	84.1	71.6	77.8	8	64.5	50.5	57.5
	Average.....54.4				Average.....62.5			
	Difference (%)..... -13.6							
3	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
	1	48.8	62.8	52.8	1	61.4	67.0	64.2
	2	78.0	83.7	80.8	2	54.7	60.1	57.4
	3	75.2	72.5	73.8	3	68.4	60.2	64.3
	4	43.4	29.8	36.6	4	42.0	43.4	42.7
	5	33.9	40.7	37.3	5	47.5	59.6	53.5
	6	62.0	55.2	58.6	6	43.1	44.5	43.8
	Average.....57.2				Average.....54.3			
	Difference (%)..... +5.3							
Average; Flats 1 - 3 ..... 54.4					Average; Flats 1 - 3...60.6			
Difference; Flats 1 - 3 (%) -10.2								

Table IIb

Area (in Square Centimeters) of Sprayed and Unsprayed Primary Leaves of Flats 4, 5, and 6, Three Days After Spraying.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
4	1	33.3	29.2	31.3	1	56.9	51.3	54.1
	2	31.9	33.3	32.6	2	72.1	63.9	68.0
	3	47.1	41.6	44.3	3	33.3	27.7	30.5
	4	19.4	26.4	22.9	4	36.1	36.1	36.1
	5	65.1	79.0	72.0	5	63.8	62.4	63.1
	6	33.3	37.5	35.4	6	18.0	18.0	18.0
	7	59.6	59.6	59.6	7	55.5	47.1	51.3
	Average.....42.6				Average.....45.9			
	Difference (%)..... -5.1							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
5	1	67.3	67.3	67.3	1	75.5	70.0	72.7
	2	55.2	55.2	55.2	2	70.0	57.9	63.9
	3	51.1	58.0	54.5	3	83.5	81.8	82.6
	4	51.1	41.8	46.4	4	63.3	55.1	59.2
	5	48.5	47.1	47.8	5	66.0	67.3	66.6
	6	90.2	90.2	90.2	6	55.2	64.6	59.9
	7	74.0	57.9	65.9	7	44.5	40.4	42.5
	Average.....61.0				Average.....63.9			
	Difference (%)..... -4.5							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
6	1	52.5	49.8	51.1	1	60.2	48.2	54.2
	2	52.5	49.8	51.1	2	42.9	45.5	44.2
	3	46.9	45.5	46.2	3	72.3	67.0	69.6
	4	38.8	40.2	39.5	4	49.1	58.5	53.8
	5	48.4	73.1	60.7	5	67.7	71.6	69.6
	6	47.8	41.2	44.5	6	51.8	55.8	53.8
	Average.....48.8				Average.....57.5			
	Difference (%)..... -15.1							
Average; Flats 4 - 6 ..... 50.9					Average; Flats 4 - 6 . 55.7			
Difference; Flats 4 - 6 (%) -8.6								

The average area of the primary leaves of the sprayed plants from all the flats was 52.7 square centimeters; that of the primary leaves of the corresponding unsprayed plants was 58.2 square centimeters. The difference in size was 9.4 percent of the area of the untreated leaves.

Effect of spraying with Bordeaux mixture upon weight. -The effect of spraying with Bordeaux mixture upon the weight of the primary leaves is shown in Tables IIIa and IIIb.

Table IIIa

Weight (in Grams) of Sprayed and Unsprayed Primary Leaves of Flats 1, 2, and 3, Seven Days After Spraying.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
1	1	.70	1.11	.905	1	1.04	1.00	1.020
	2	.85	1.08	.965	2	1.55	1.66	1.605
	3	1.05	1.50	1.275	3	1.07	1.26	1.165
	4	1.06	1.14	1.100	4	1.13	1.12	1.125
	5	1.20	1.02	1.110	5	1.04	1.10	1.070
	6	1.05	1.12	1.085	6	.96	.95	.955
	7	.42	.62	.520	7	1.34	1.70	1.520
	Average..... .994				Average..... 1.208			
	Difference (%).....-16.9							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
2	1	1.11	1.36	1.235	1	.77	.77	.770
	2	1.21	1.04	1.125	2	1.77	1.64	1.705
	3	1.30	1.53	1.415	3	1.45	1.37	1.410
	4	.45	.36	.405	4	.62	.62	.620
	5	.62	.72	.607	5	1.69	1.77	1.730
	6	1.17	1.10	1.135	6	1.47	1.60	1.535
	7	.68	.68	.680	7	1.40	1.23	1.315
	8	1.650	1.380	1.515	8	1.19	.97	1.080
	Average..... 1.023				Average.....1.270			
	Difference (%)..... -19.4							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
3	1	1.00	1.17	1.085	1	1.23	1.35	1.290
	2	1.47	1.54	1.505	2	1.06	1.19	1.120
	3	1.36	1.35	1.355	3	1.31	1.16	1.235
	4	.75	.48	.615	4	.81	.81	.810
	5	.63	.75	.690	5	.86	1.10	.980
	6	1.20	1.10	1.150	6	.80	.88	.840
	Average..... 1.067				Average..... 1.046			
	Difference (%)..... 1.9							
Average; Flats 1 - 3..... 1.023					Average; Flats 1 - 3..1.186			
Difference; Flats 1 - 3(%) -13.8								

Table IIIb

Weight (in Grams) of Sprayed and Unsprayed Primary Leaves of Flats 4, 5, and 6, Three Days After Spraying.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
4	1	.51	.43	.470	1	.85	.79	.820
	2	.48	.52	.500	2	1.11	.95	1.030
	3	.71	.65	.680	3	.58	.45	.515
	4	.33	.40	.365	4	.50	.52	.510
	5	1.14	1.45	1.295	5	1.07	1.05	1.060
	6	.55	.57	.560	6	.26	.26	.260
	7	1.00	1.02	1.010	7	.98	.74	.860
	Average..... .700				Average..... .722			
	Difference (%)..... -3.4							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
5	1	1.23	1.26	1.225	1	1.35	1.27	1.310
	2	1.10	1.06	1.080	2	1.34	.93	1.135
	3	.87	1.04	1.455	3	1.68	1.53	1.605
	4	.93	.82	.875	4	1.08	1.00	1.040
	5	.88	.86	.870	5	1.37	1.50	1.435
	6	1.73	1.78	1.755	6	.98	1.22	1.100
	7	1.34	1.02	1.180	7	.85	.80	.825
	Average.....1.205				Average.....1.207			
	Difference (%)..... -0.2							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
6	1	.95	.99	.970	1	1.18	.90	1.040
	2	1.06	.98	1.020	2	.83	.89	.860
	3	.89	.87	.880	3	1.32	1.25	1.285
	4	.72	.73	.725	4	1.02	1.20	1.110
	5	1.06	1.39	1.225	5	1.31	1.42	1.365
	6	.88	.72	.800	6	.98	1.02	1.000
	Average..... .903				Average..... 1.110			
	Difference (%).....-18.6							
Average; Flats 4 - 6..... .947					Average; Flats 4 - 6 1.008			
Difference; Flats 4 - 6 (%) -6.0								

The average weight of the sprayed leaves from each flat was less than that of the corresponding unsprayed leaves in all cases, except Flat 3. The average weight of the primary leaves of the sprayed plants from all the flats was 0.986 gram; that of the primary leaves of the corresponding unsprayed plants was 1.099 gram. The difference in weight was 10.3 percent of the weight of the unsprayed leaves.

Comparison of fresh weight of sprayed and unsprayed leaves per square centimeter. - Because of the fact that the sprayed leaves differed in total area and total fresh weight from the unsprayed leaves to about an equal degree, there has been, considering the data as a whole, no change in the weight per unit area. There has been, however, a variation from flat to flat in the weight per unit area. A consideration of Tables IVa and IVb will show that spraying with Bordeaux mixture has not exerted a consistent effect upon the weight per unit area, i.e., the sprayed and unsprayed leaves are, on the average, of the same texture.

Table IVa

Fresh Weight (in Milligrams) of Sprayed and Unsprayed Primary Leaves of Flats 1, 2, and 3, Seven Days After Spraying.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
1	1	17.5	19.0	18.4	1	17.9	17.6	17.8
	2	21.2	19.0	20.1	2	19.3	19.7	19.5
	3	20.3	18.2	19.3	3	19.3	20.2	19.7
	4	20.8	18.3	19.5	4	17.8	18.0	17.9
	5	20.2	17.2	18.7	5	17.7	18.1	17.9
	6	20.0	18.5	19.2	6	18.8	18.6	18.7
	7	20.2	18.7	19.4	7	19.4	20.3	19.8
	Average.....19.2				Average.....18.8			
	Difference (%)..... +2.1							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
2	1	17.9	20.5	19.2	1	19.9	20.4	20.1
	2	17.5	17.5	17.5	2	25.4	23.7	24.5
	3	18.4	20.4	19.4	3	20.2	19.1	19.6
	4	18.4	15.1	16.7	4	18.0	16.6	17.3
	5	18.0	18.6	18.3	5	21.5	21.4	21.4
	6	19.5	19.3	19.4	6	17.8	19.7	18.8
	7	18.3	18.3	18.3	7	20.0	21.5	20.7
	8	19.6	19.3	19.5	8	18.5	19.2	18.8
	Average.....18.5				Average.....20.2			
	Difference (%)..... -8.4							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
3	1	20.5	18.6	19.5	1	20.0	20.2	20.1
	2	18.8	18.4	18.6	2	19.4	19.8	19.6
	3	18.1	18.6	18.3	3	19.2	19.3	19.2
	4	17.3	16.1	16.7	4	19.3	18.7	19.5
	5	18.6	18.4	18.5	5	18.1	18.5	18.3
	6	19.4	19.9	19.7	6	18.5	19.8	19.1
	Average.....18.6				Average.....19.2			
	Difference (%)..... -3.1							
Average; Flats 1 - 3				18.8	Average; Flats 1 - 3			19.4
Difference; Flats 1-3 (%).. -3.1								

Table IVb

Fresh Weight (in Milligrams) of Sprayed and Unsprayed Primary Leaves of Flats 4, 5, and 6, Three Days After Spraying.

Flat	Sprayed				Unsprayed			
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
4	1	15.3	14.7	15.0	1	14.9	15.4	15.1
	2	15.1	15.6	15.3	2	15.4	14.9	15.1
	3	15.1	15.6	15.3	3	17.4	16.3	16.7
	4	17.0	15.8	16.4	4	13.8	14.4	14.1
	5	17.5	18.4	17.9	5	16.9	16.9	16.9
	6	16.5	15.2	15.9	6	14.4	14.4	14.4
	7	16.8	17.1	16.9	7	17.6	15.7	16.7
	Average.....16.1				Average.....15.6			
	Difference (%)..... +3.2							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
5	1	18.3	18.2	18.2	1	17.9	18.1	18.0
	2	19.9	19.2	19.5	2	19.1	16.1	17.6
	3	17.0	18.0	17.5	3	20.2	18.7	19.4
	4	18.2	19.6	18.9	4	17.1	18.2	17.6
	5	18.1	18.2	18.1	5	20.8	22.3	21.6
	6	19.2	19.7	19.4	6	17.7	18.9	18.4
	7	18.1	17.6	17.8	7	19.1	19.8	19.4
	Average.....18.5				Average.....18.8			
	Difference (%)..... -1.6							
	Plant	Primary Leaves		Ave.	Plant	Primary Leaves		Ave.
6	1	18.1	19.9	19.0	1	19.6	18.7	19.6
	2	21.2	19.7	20.4	2	19.4	19.6	19.5
	3	19.0	19.1	19.0	3	18.3	18.7	18.5
	4	18.8	18.2	18.5	4	20.8	20.5	20.6
	5	21.9	19.0	20.4	5	19.4	19.8	19.6
	6	18.4	17.5	17.9	6	13.7	14.0	13.4
	Average.....19.2				Average.....18.5			
	Difference (%)..... +3.8							
Average; Flats 4 - 6..... 17.9					Average; Flats 4 - 6.. 17.6			
Difference; Flats 4 - 6 (%). +1.7								

Variation in chlorophyll content per half-flat units. - The average chlorophyll content of the primary leaf of the average sprayed and unsprayed plant is shown in Table V.

Table V

Average Chlorophyll Content (in Milligrams) of the Average Primary Leaf of Each Plant.

Flat	1	2	3	4	5	6	Average
Sprayed	2.13	2.05	2.17	1.61	2.08	1.80	1.98
Unsprayed	2.11	2.50	2.00	1.57	1.94	2.05	2.03

The table shows no constant difference between the chlorophyll content of the average sprayed and unsprayed leaf; the average chlorophyll content for the sprayed and unsprayed leaves of the six flats is very nearly the same. By taking into consideration the independent variation in weight per unit area, as indicated by half-flat averages, and the variation in area induced by spraying, these results can be interpreted.

It has been shown above that spraying has resulted in a reduction in the average area and average fresh weight of the primary leaves. Although the average areas and weights of the sprayed leaves are approximately ten percent less than the average areas and weights of unsprayed leaves, the average chlorophyll content of the sprayed leaves, as shown by Table V, is approximately the same as that of the unsprayed leaves. The difference in the effect of spraying upon the chlorophyll content and area and weight very plainly indicates a greater amount of chlorophyll in a unit area or unit

weight of sprayed leaf than in the same area or weight of unsprayed leaf. The constant variation in this direction is seen in Table VI and Table VII.

Table VI

Chlorophyll Content (in Milligrams) per Square Centimeter.

Flat	1	2	3	4	5	6	Average
Sprayed	.0427	.0376	.0378	.0339	.0360	.0364	.0374
Unsprayed	.0329	.0402	.0369	.0286	.0305	.0354	.0341
Difference (%)	+29.8	-6.5	+2.4	+18.5	+18.0	+2.7	+9.7

Table VII

Chlorophyll Content (in Milligrams) per Gram Fresh Leaves

Flat	1	2	3	4	5	6	Average
Sprayed	2.14	2.00	2.04	2.30	1.82	2.00	2.05
Unsprayed	1.75	1.97	1.96	2.17	1.61	1.86	1.89
Difference (%)	+22.3	+1.5	+4.1	+6.0	+13.0	+7.5	+8.5

Reference to Table IVa will show the exceptionally high weight per unit area of the unsprayed leaves from Flat 2, which is doubtless a cause of the exceptionally high chlorophyll content per unit area and unit weight of the unsprayed leaves from this one flat.

IV Relation Between the Age of the Primary Leaf and its Chlorophyll Content

Willstätter and Stoll (1915) have shown that the chlorophyll content of leaves which have just attained full size is much greater per square centimeter than that of very young leaves. After reaching full size the leaf shows a decrease in chlorophyll content. In the course of my experiments the question of the effect of age naturally arose. It was desirable to know whether there had been a natural increase or decrease between the periods of spraying and harvesting, and whether the exact stage of development at harvesting was a matter of great importance. It was thought that there might be a gradual decrease in the chlorophyll content of the primary leaves as younger and photosynthetically more active leaves (Willstatter and Stoll, 1915) developed. Further, in view of the fact that Palladin (1891) has shown that etiolated leaves do not regain their chlorophyll content in the absence of a supply of a soluble carbohydrate, a relation might exist between the chlorophyll content of the primary leaves and the presence of the cotyledons. The chlorophyll content of the primary leaves before and after the shedding of the cotyledons was not without interest.

The chlorophyll content of the primary leaves was, therefore, determined at the following four stages of development: - (1) "Age A", immediately after the primary leaves had unfolded; (2) "Age B", when the cotyledons were being shed; (3) "Age C", when the primary leaves had attained a considerable size, but while their area still constituted the greater part of the photosynthetic surface (this state occurs in a very few days after "Age B", in this case

this age was reached four days after "Age B"); (4) "Age D", when the primary leaves constituted only a small part of the leaf surface. (In this case seventeen days were allowed to elapse between "Age C" and "Age D"). The results are shown in Table VIII.

Table VIII

Chlorophyll Content of Primary Leaves at Various Ages.

Age	Ave. Leaf Area (Sq.CM.)	Ave. Fresh Weight per (Sq.Cm.(Grams)	Chlorophyll Mg. per Sq. Cm.	Chlorophyll Mg. per Gram Fresh Weight.
"A"	11.7	.0244	.0323	1.32
"B"	32.3	.0185	.0412	2.22
"C"	53.9	.0201	.0398	1.96
"D"	73.0	.0205	.0389	1.89

The data for "Age A" were obtained by measuring and weighing the primary leaves of thirty-two plants; the primary leaves from twenty plants were used to obtain the data for "Age B"; sixteen plants were used for "Age C"; and twenty plants for "Age D".

It is seen that from "Age C", which represented the stage of development when the records were made in the experiments on the effect of spraying with Bordeaux mixture, to "Age D", when the plants had attained approximately their full size, there was very little decrease in the amount of chlorophyll per unit area or unit of fresh weight, notwithstanding the unfolding of a large area of new foliage. It is also seen that the highest chlorophyll content was attained at "Age B", that is, when the cotyledons were being shed.

V Effect of the Removal of the Growing Bud Above the Primary Leaves upon the Chlorophyll Content of the Primary Leaves

It has been shown in the report of the previous experiment that the chlorophyll content was highest just at the period of the shedding of the cotyledons. In the light of Palladin's experiments it may be supposed that this was related to the carbohydrate supply. Therefore, an experiment was carried out to determine the effect of the removal of the growing bud on the chlorophyll content of the primary leaves. The growing bud of half of the plants of three flats were removed when the plants had reached "Age A". Measurements and weights were taken, and the chlorophyll content was determined, at "Age C". The results of this experiment show a marked increase in the comparative area and weight per unit area of the primary leaves of the plants from which the growing bud had been removed, and a disproportionally greater increase in the chlorophyll content. The results are presented in Table IX.

Table IX

Effect upon the Primary Leaf of the Removal of the Growing Bud.

Flat	Plants	Area, Sq. Cm.	Weight of Sq. Cm. of Fresh Leaf (Grams)	Chlorophyll (in Mg.) per Gram Fresh Leaf
1	Normal	37.7	.0184	1.74
	Growing Bud Removed	59.6	.0226	2.00
	Difference (%)	+58.2	+22.8	+14.9
2	Normal	41.5	.0186	1.90
	Growing Bud Removed	55.6	.0238	2.17
	Difference (%)	+34.0	+27.9	+14.2
3	Normal	46.2	.0201	1.91
	Growing Bud Removed	63.3	.0253	2.10
	Difference (%)	+37.0	+25.8	+9.9
Ave.	Normal	41.8	.0190	1.85
	Growing Bud Removed	59.5	.0239	2.09
	Difference (%)	+42.5	+25.8	+13.0

When the plants were still growing it was thought that the greener color of the primary leaves following the removal of the growing bud might be due solely to their obviously greater thickness. The chlorophyll content per unit of fresh weight did not, however, remain constant, as would have been necessary in such a case, but

increased, on the average, thirteen percent.

VI The Cumulative Effect of Bordeaux Mixture as Indicated by Increase in the Length of the Primary Leaf

It has been shown in the results reported under the heading, "The Effect of Bordeaux Mixture", that there was a marked difference in the area and chlorophyll content attained by sprayed and unsprayed leaves. This effect of the application of Bordeaux mixture was manifest in the measurements and determinations of chlorophyll made three days after the application of the mixture. It was further shown that about an equal effect was to be observed if the growing leaves were measured, weighed, and analyzed for chlorophyll, seven days after spraying. The rapidity with which the effect is brought about is not surprising in view of the rapid growth of the leaf between ages "A" and "B", when the spray was applied, and "Age C", when the results of spraying were determined. A quickly manifested difference in the size and chlorophyll content of sprayed and unsprayed leaves was to be anticipated in view of the quickly manifested effect of Bordeaux mixture upon transpiration. (Martin, 1916). The following series of experiments was undertaken with a view to determining (1) how soon after spraying the effect upon the relative increase in the size of the leaves was to be observed, and (2) whether or not the retardation in growth caused by the application of Bordeaux mixture was permanent or was gradually overcome with the result that the sprayed leaves finally attained the size of the unsprayed leaves.

Five flats were planted to beans, one flat at a time, at intervals of from one to five days. The total period of planting covered eighteen days, and the plants were exposed, on that account, to slight variations due to slight changes in weather conditions. As soon as the primary leaves had unfolded, the plants in one-half of each flat were sprayed with Bordeaux mixture. At the time of spraying, records were made of the length of the primary leaves of the sprayed and unsprayed plants. The length of the leaves was determined at intervals of two, four, eight, twelve, and fourteen days after spraying. It was found that growth in length of sprayed and unsprayed leaves did not occur after the twelfth day, so that the records presented in Table X are for the twelve-day period only.

Table X

Effect of an Application of Bordeaux Mixture as Shown by a Decreased Growth in Length (Centimeters).

Days After Spraying	Treatment	Flat Number					Percentage Difference of Sprayed Leaves
		1	2	3	4	5	
--	Sprayed Unsprayed	6.1 6.1	4.3 4.1	4.6 4.4	3.9 3.6	3.3 3.4	3.6
2	Sprayed Unsprayed	6.8 7.1	5.4 5.6	5.9 5.9	5.8 5.5	4.8 4.9	-1.0
4	Sprayed Unsprayed	7.2 7.7	6.4 6.5	6.8 6.9	6.6 6.9	5.7 5.6	-2.8
8	Sprayed Unsprayed	7.6 8.2	7.0 7.2	7.6 7.8	7.7 7.8	5.9 6.3	-4.2
12	Sprayed Unsprayed	7.7 8.3	7.3 7.4	7.7 7.9	7.9 8.3	6.3 6.5	-4.1

It is seen, under the column headed, "Percentage Difference of Sprayed Leaves", that a decided effect had been produced in the first two days after spraying, and that the difference increased until the eighth day; from the eighth to the twelfth day the difference remained constant. It is also to be observed that the sprayed leaves did not attain the size of the unsprayed leaves. An examination of the figures given in the first and fourth pairs of horizontal columns, which give the average lengths of the leaves at the beginning of the experiment and after eight days, will show that, although the average length of the leaves subsequently sprayed was in three flats greater than the average length of those serving as checks, the average length of the sprayed leaves eight days after

spraying was, in every case, less than that of the unsprayed leaves.

VII Discussion

Methods of analysis. - It was found that the shade of color of the eighty-five percent acetone extracts from the leaf-powders analyzed for their chlorophyll content matched very closely the shade of color of the eighty-five percent acetone extract from the normal leaf-powder used as a standard. If this had not been the case other methods of analysis would have been necessary. "Die Rohchlorophyllösung enthält die vier Pigmente von verschiedener Farbe und Farbintensität in Mengenverhältnissen, die vom Lösungsmittel beeinflusst werden und die von der Pflanzenart und sogar von der Ernte einer und derselben Pflanze abhängig sein können. Annähernd gleich ist das Mengenverhältnis der Komponenten bei den Extrakten einer Pflanze; man kann daher durch den Vergleich derselben die relative Bestimmung ihres Chlorophyllgehaltes ausführen. Für die Untersuchung von Extrakten ungleicher Farbnuance, also z.B. aus verschiedenen Pflanzen, ist es erforderlich, die Farbstoffe durch Verseifung mit Alkali in die indifferenten gelben und in die Alkalisalze der grünen Pigmente zu trennen. Die erhaltenen Chlorophyllinlösungen ermöglichen die relative Bestimmung des Farbwertes und ferner erlaubt ihr Vergleich mit einer alkoholischen Lösung von bekanntem Chlorophyllgehalt die absolute Bestimmung des Farbstoffgehalts." (Willstatter and Stoll, 1913, pp. 78 - 79).

Probable relation between photosynthesis and the increased chlorophyll content. - It has been shown that the chlorophyll content per unit area and per unit of fresh weight has been increased

by spraying with Bordeaux mixture, and by the removal of the bud above the primary leaves. It is altogether likely, at least in the case of the increased chlorophyll content as a result of spraying, that the rate of photosynthesis has been increased in the same proportion. Recent results on the relation between the chlorophyll content and the rate of photosynthesis support this view. Willstätter and Stoll (1915) conclude from their investigations of the photosynthetic activity of variously colored leaves that the rate of photosynthesis varies with the chlorophyll content in the case of normal leaves, although in etiolated leaves, very young leaves, and chlorotic leaves the rate of photosynthesis is greater than that indicated by the chlorophyll content. Plester (1912) concludes that structure is a factor in the rate of photosynthesis of varieties of the same species differing in their chlorophyll content, and Rose (1913, p. 105) arrives at a similar conclusion as the result of his work on the photosynthetic rate manifested by leaves developed under different conditions of illumination. In the experiments above reported on the effects of Bordeaux mixture no change has taken place in the leaf structure, so far as a change would be indicated by a change in the weight per unit area. No direct experiments on the photosynthetic rate of sprayed leaves were carried out since that is one of a series of topics outlined for further experiment.

Environmental conditions. - The conditions for the growth of the plants used in this experiment were extremely favorable to rapid and normal development. Extremes were avoided and it is probably due to the care thus exercised that injuries such as those

reported by Frank and Krüger (1893) were not observed. These authors reported a severe Bordeaux injury to the foliage of potatoes as the result of adverse conditions, including very high temperature. Bain (1902) states that high temperature is a factor in the physiological response of peach foliage to copper salts, as manifested in both increased assimilation and increased injury. Crandall (1909) states that low vitality is a factor in the production of injury to the foliage of the apple following the application of Bordeaux mixture. The freedom of the plants from injuries may have been due also to the fact that the foliage was not wetted, since Bain states that injury to the peach occurs only as a result of wetting. Schander (1904) has shown, however, that bean foliage can be injured by Bordeaux mixture without the presence of liquid water. Although it is possible that under certain conditions the foliage would not respond, in any way, to the application of Bordeaux mixture, such a lack of response is not probable in view of the very general response to copper salts of plants of very widely separated species.

VIII Summary

1. The primary leaves of the bean sprayed with Bordeaux mixture do not grow to the size of unsprayed leaves.

2. A retardation in growth is manifested very soon after the spray is applied.

3. The chlorophyll content per unit area of the primary leaves of the bean is slightly increased by spraying with Bordeaux mixture.

4. The chlorophyll content per unit area of the primary

foliage leaves of the bean decreases as the leaves develop after the shedding of the cotyledons.

5. The chlorophyll content per unit area of the very young primary leaves of the bean is lower than that of the primary leaves of the bean just after the shedding of the cotyledons.

6. The primary leaves of the bean increase in area and in weight per unit area following the removal of the bud above the primary leaves.

7. This increase in area and weight per unit area is accompanied by a disproportionately greater increase in the chlorophyll content per unit area.

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Figure I

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X Vita

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1912-1918.	Associate Chemist in Horticulture, University of Illinois.
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